BACTERIAL SOURCE TRACKING

US EPA GMP
Policy Review Board Meeting
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BACTERIAL SOURCE TRACKING

National Focus
(What is happening in the United States?)

Mississippi Focus
(What is happening in Mississippi/USM?)

What will BST mean in the future?
(What the principal questions related to this technology?

(How can we improve BST?

(How can the USEPA GMP help in this effort?)

BACTERIAL SOURCE TRACKING
A critical need for States bordering the Gulf of Mexico

A National Thrust:
- The Problem
  - Animal origin of fecal pollution of water
- The Need
  - Identify methods & indicators
  - Define local, State, Regional and national value
- The Solution
  - Effective Bacterial Source Tracking Databases

Specific Concerns:
- TMDL surveys
- Beach Closing
- Effect on Economic Development
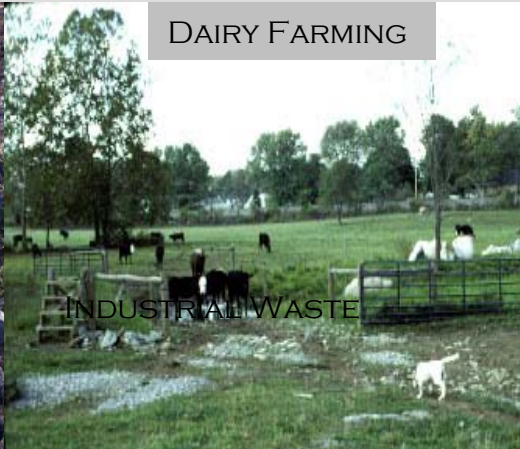- Effect on Agricultural Activities
- Health of Population
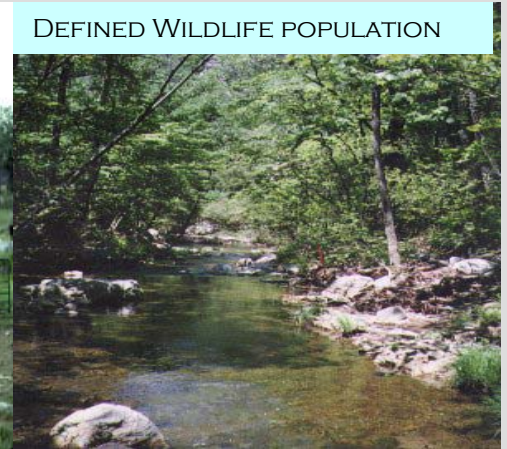
BACTERIAL SOURCE TRACKING

POINT SOURCES

BACTERIAL SOURCE TRACKING
Non-Point Sources

MARINE FISH

WILD ANIMALS

PLANT MATTER

SHORE BIRDS

© Don Getty
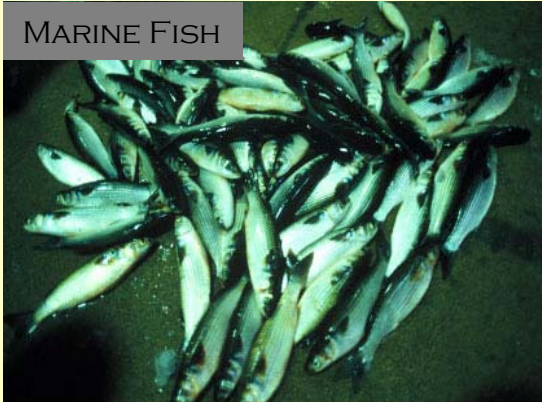
SEDIMENTS

MIGRATING BIRDS

Sooty Shearwaters, Colac Bay, New Zealand, copyright Duncan Poyser

BACTERIAL SOURCE TRACKING - Needs

FC

↓

EC/EN

↓

EC/EN

FROM

SPECIFIC ANIMAL

Standard coliform measures cannot determine the source of fecal pollution of Coastal waters

accurate risk assessment, effective remediation and valid TMDL analysis

Unknown but credible economic impact

As the coast population grows the likelihood of closures increases. Need to pinpoint sources.

Evaluate relationships: indicator organisms are not confined to warm bloodied animals but are found in plants, invertebrates, and numerous other sources.

BACTERIAL SOURCE TRACKING - Benefits

Define the animal source of pollution of coastal waters

Availability of new regulatory information to be used by state and federal agencies during cases in which counts of indicator bacteria are insufficient or unable to determine the source of pollution

Allow regulatory agencies data representative of the level of contamination, the animal population involved, and the ability to develop remedial measures to decrease contamination

Create a relationship of database to larger regional/ watershed projects and to the efforts of the state to define watersheds that affect coastal water quality
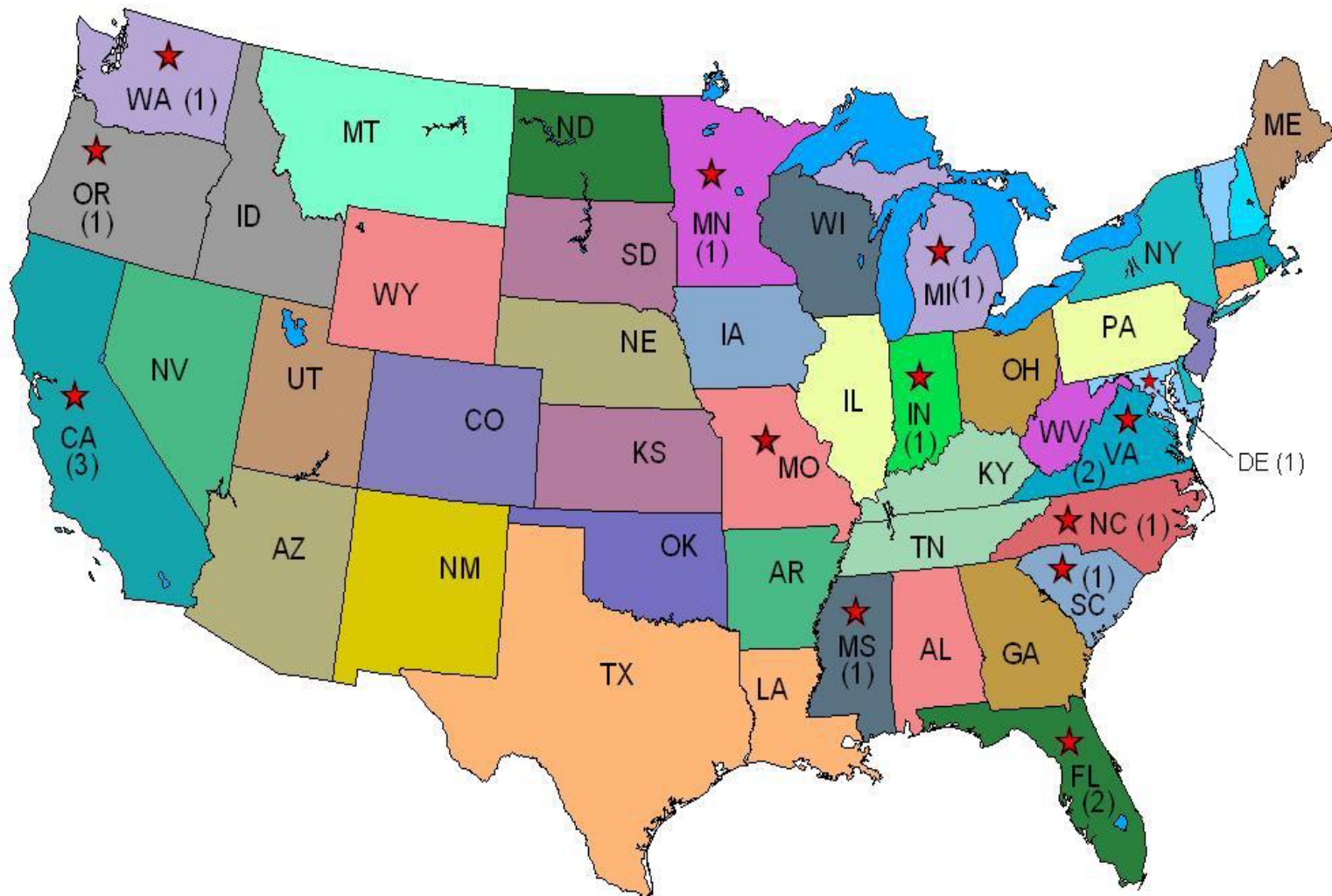
BACTERIAL SOURCE TRACKING - Agencies

- **Agencies Interest is Extensive**
  - US EPA
    - USEPA GMP
  - USGS
  - USDA
  - NIEH
  - State Departments of Agriculture
  - State Departments of Environmental Quality
  - State Departments of Marine Resources
  - State Departments of Health
  - State Departments of Wildlife and Fisheries
  - Harrison County Sand Beach Authority
  - Southern California Coastal Water Resources Project Authority (Sccwrpa)

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- Southern California Coastal Water Resources Authority
  - Began as Regional study
  - First National study (on going)
  - Focus on methods and library value
  - Multi-University project

- Need for Gulf Wide Initiative (New GMP initiative at USM)
  - Similar environments
  - Increasing Populations
  - Waters are threatened
  - Excellent area of study
  - Expandable to all coastal states

BACTERIAL SOURCE TRACKING

Bacterial Source Tracking - Methods

Mechanisms of Bacterial Source Tracking

Ribotyping, PCR (rep, ERIC, BOX)

RAPD, Pulse Field Gel Electrophoresis (PFGE)

Antibiotic Resistance Analysis

Coliphage tracking, Bacteroides analysis

Enterotoxin genes as biomarkers

Enterovirus and Adenovirus isolation

Southern Calif. Coastal Water Research Project

    Testing methods of source tracking

    Comparing indicator organisms

    Examining database analysis

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- The Mississippi/USM Initiative:

  - Build a library of animal samples and isolates
  - Focus on Animals most likely to affect Mississippi waters
  - Utilize the most important indicator species
  - Fingerprint isolates using the best methods
    - Reliability
    - Statistically significant measures
  - Apply the best analysis software
  - Test the protocols with blind known isolates
  - Refine the protocols as necessary
  - Apply the methods to unknown samples from Mississippi watersheds
  - USM participation in the National Sccwrpa study

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USM Investigators

R.D. Ellender, Shiao Wang, Bob Middlebrooks

Dawn Rebarchik, D. Jay Grimes

Collaborators/Interested Partners

• USEPA Gulf of Mexico Program (F)

• MS Department of Agriculture (F)(P)

• MS Department of Environmental Quality (F)(P)

• NOAA Coastal Assistance Impact Program (F)

• Southern California Coastal Water Resources Authority (F)

• MS Department of Wildlife, Fisheries and Parks (S)

• MS Department of Marine Resources(P)

• MS Department of Health

F funding; P pending, S samples

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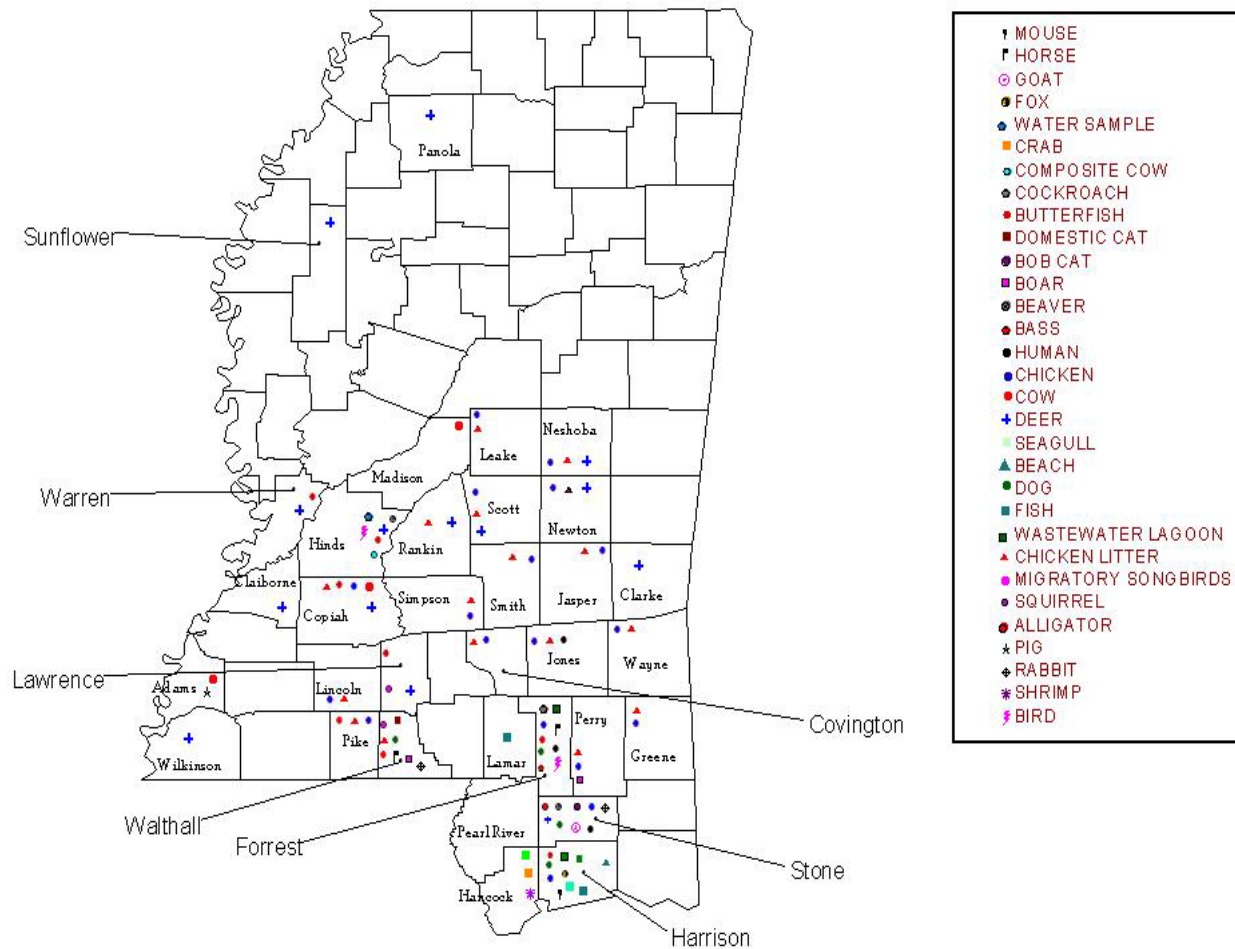
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Sample collection initiated September 2001

983 samples collected 9/01-11/02

E. coli and Enterococcus Isolates collected same period

4721 isolates in database; approx. =#s of EC&EN

Samples from 30 different animals; 2 composite sample

Approximately 70% of our samples are from humans, cow, deer and chickens.
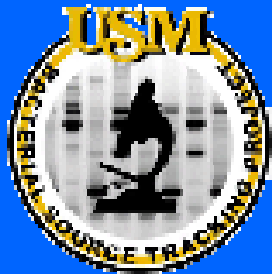
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Isolation using *EPA Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and E.coli March 2000 (EPA/821/R-97/004)*

DNA Fingerprinting

repPCR, BOX-PCR and Pulse Field Gel Electrophoresis (PFGE)
ARA analysis
Cluster Analysis

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Conditions:

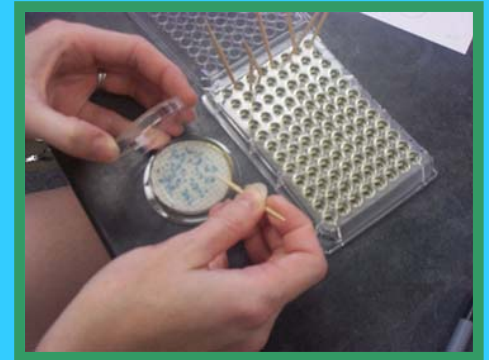*Archive*: Samples and isolates into FBS/10% DMSO

Bacterial Isolation:

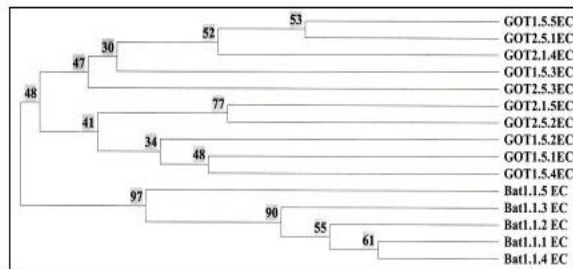E.coli: mTEC modified 44.5°C; Enterococci: mEI agar; 41°C
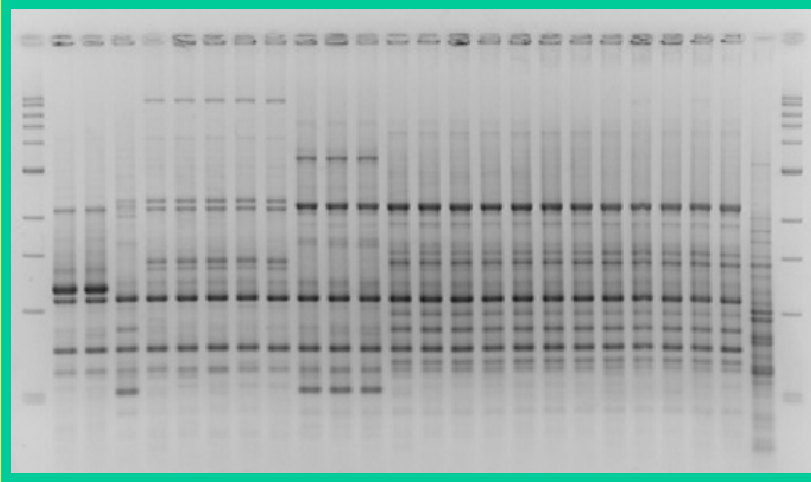
Transfer colonies to BHI broth tube and archive

DNA Analysis

Whole cells washed. PCR – DNA amplified followed by gel analysis; PFGE – DNA digested using restriction enzymes followed by gel analysis and documentation (pic file).

Cluster Analysis: Applied Maths's BioNumerics

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→ Gel Fingerprint

↓

Digitize isolate band pattern

↓

Identify/Confirm bands

↓

Place band data into BioNumerics

↓

Compare Isolates using statistical

strategies (dendograms; cluster

analysis)

←

Dendrogram showing the relatedness of the E coli isolates

BACTERIAL SOURCE TRACKING

File   Edit   View   Favorites   Tools   Help

Back | Search Favorites Media

Address https://www.quickbase.com/db/7ss5iemn?act=ListAll Links

MY QUICKBASE – SIGN OUT – SUP

BST Samples

MAIN - ADD RECORD - LIST - VIEWS

Find →

Advanced Find

List All, by Source of sample

395 records in view
1 to 132 displayed

These Records - Spreadsheet - Print-friendly - Refine View - Clear Flags

	Source of sample	Location from which sample was obtained	Sample collected by	Date sample collected	Sample Name	Sample ID #	Database Link	Storage Location	EC isolates obtained	EN isolates obtained
edit view	Alligator	Red Creek WMA	Dr. Ellender	01-17-2002	ALL 1-1	1288	1288		0	0
edit view	BASS	BIG BAY LAKE WEST OF HATTIESBURG	RDE	03-16-2002	BAS 6	1243	1243		0	0
edit view	Bass	Big Bay Lake	Dr. Ellender	11-24-2001	BAS 4	1265	1265		0	0
edit view	Bass	Big Bay Lake	Dr. Ellender	11-24-2001	BAS 3	1264	1264		0	0
edit view	Bass	Big Bay Lake	Dr. Ellender	11-24-2001	BAS 2	1263	1263		0	0
edit view	Bass	Big Bay Lake	Dr. Ellender	11-24-2001	BAS 1	1262	1262		0	0
edit view	BASS	BIG BAY LAKE WEST OF	RDE	03-16-2002	BAS 8	1245	1245		0	0

Internet

To view this and other BST databases :

HTTP://WWW.QUICKBASE.COM; REGISTER; SHIAO.WANG@USM.EDU ; REQUEST PERMISSION TO VIEW SITE

BACTERIAL SOURCE TRACKING

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Jackknife Analysis

- A type of cluster analysis to determine the stability of defined groups, in this case, *E. coli* or enterococci from known host species.

- DNA fingerprints of known isolates are compared to entries among the different groups and average similarities calculated. Done for all entries.

- The percentage of cases that entries are identified to the group they are assigned to is a measure of the internal stability of that group.

- The higher the percentage similarity – the more reliable the species assignment.

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JACKKNIFE ANALYSIS BOX-PCR VS REP-PCR FOR *E. COLI*

BOX-PCR

	Human	Cow	Deer
Human	14 %	37 %	49 %
Cow	0 %	64 %	36 %
Deer	0 %	0 %	100 %

Isolates: 155 human, 212 cow and 45 deer.

REP-PCR

	Human	Cow	Deer
Human	75 %	20 %	5%
Cow	7 %	90 %	3 %
Deer	2 %	36 %	62 %

Isolates: 115 human, 198 cow and 50 deer.

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- ANTIBIOTIC RESISTANCE ANALYSIS (ARA) IS ALSO BEING PERFORMED ON ISOLATES OF E. COLI AND ENTEROCOCCI.
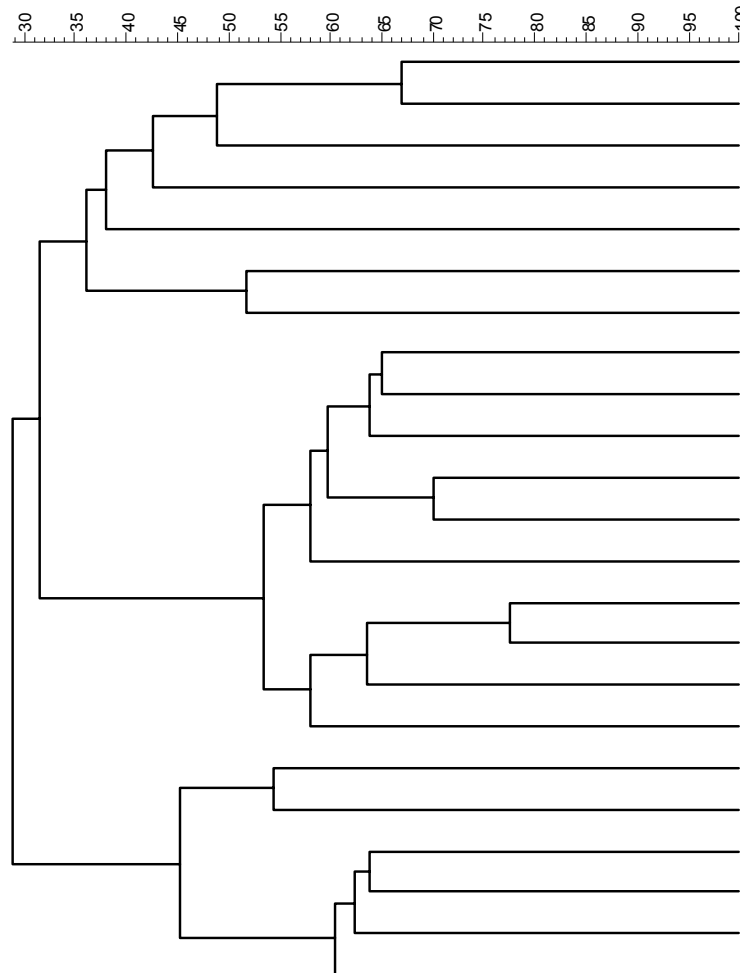
	ANIMAL	HUMAN
E. COLI (374)		
ANIMAL	90.1	9.9
HUMAN	0	100
ENTEROCOCCI (686)		
ANIMAL	95.0	5.0
HUMAN	7.7	92.3

BACTERIAL SOURCE TRACKING

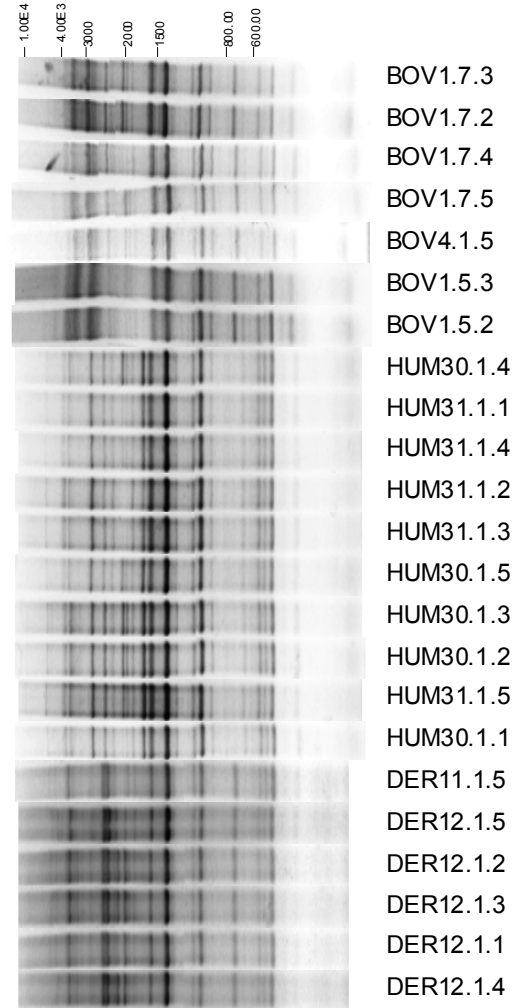

Jaccard(fuzzy)(area) (Opt:3.00%) (Tol 3.0%-3.0%) (H>0.0% S>0.0%) [0.0%-100.0%]
REP-PCR

REP-PCR

Bacterial Source Tracking – Summary
What are we going in the next three years?

- Increase the number of database isolates to 15,000 in the next 3 years; move toward a statewide database.

- Integrate GIS into statewide sampling plan

- Utilize the most discriminating fingerprinting method(s) that current research can suggest; be part of the selection process

- Increased collaboration with other state and national researchers; develop the Gulf States BST Initiative

Bacterial Source Tracking – Summary
What is the promise of the technology?

- Excellent chance of success = Automation

  - Integrate Typhoon DNA analysis
  - Automate the PCR reaction
  - Develop BST Robotics as a means of reducing manual labor, allowing faster sample processing and data analysis, producing better coverage of the State's water resources.
  - Additional studies in which technology is taken "to the field" to demonstrate the value of the technology.

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- What might be the role of the USEPA GMP in the future of BST?
  - create a research focus in all Gulf States

  - Assist formation of the Center for BST research to exist for all Gulf states to serve as a processing center, data repository and analysis for any state wishing to submit a sample — nominal fee — Center to also setup best methods of doing BST for everyone

  - Political — Gulf wide BST research network

BACTERIAL SOURCE TRACKING

Thank you for this opportunity

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